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A Peptoid-based targeted Drug delivery system for the treatment of Metastatic cancer

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A Peptoid-Based Targeted Drug Delivery System for the Treatment of Metastatic Cancer

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by

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Abstract

Development of a targeted drug delivery system is a critical step in the effort to improve cancer treatments. Such a system would greatly reduce the harmful side effects of chemotherapy by delivering toxic drugs directly to cancerous cells. Peptoids—synthetic compounds that can be easily produced from readily available amine monomers—have great potential for use in targeted drug delivery. This project aimed to develop peptoids that would bind to specific proteins expressed on the surface of cancer cells. These peptoids could be combined into a complex that would bind to the proteins with an even greater affinity than the individual compounds. The peptoid complexes would serve as a targeting system for chemotherapeutic drugs, delivering them directly to cancer cells, thereby preventing healthy tissues from being harmed. The specific membrane proteins that are being targeted are lectin-like oxidized low-density lipoprotein receptors (LOX-1) and receptors for advanced glycation end-products (RAGE). Both LOX-1 and RAGE are expressed in higher concentrations on cancerous cells than on non-cancerous ones.

A combinatorial peptoid library was created using five side chains that have been identified as being suitable for this application. Each peptoid is six monomers long, resulting in a theoretical library diversity of 15,625 different compounds. A small sample of the library was screened to qualitatively identify peptoids with the highest binding affinity for LOX-1. To determine the sequences (chemical structures) of the peptoids that displayed high LOX-1, an automated peptide sequencer was utilized. Unfortunately, the time to work through the technicalities of

the sequencing process exceeded the length of the project-to-date. However, progress was made and the sequences may be determined in the near future. Eventually the process will be repeated to determine RAGE-binding peptoids and the peptoids will be combined in a chemotherapy drug targeting system.

Introduction

It is often acknowledged that the side effects of chemotherapy treatment can be as unpleasant as the cancer itself. These unwanted side effects are caused because the toxic drugs used to destroy metastasized cancer cells are delivered globally within the body, causing harm to both cancerous and healthy tissues alike [1]. As such, much work has gone towards the development of so-called targeted treatments for cancer. The basic idea behind targeted chemotherapy treatments is that if anti-cancer drugs can be delivered directly to cancer cells while bypassing healthy tissues, then the efficacy of the drugs will increase and their harmful side effects will decrease.

One potential means of targeting therapeutics to cancer cells is to encapsulate anti-cancer drugs within a microscopic structure, such as a microbubble, that contains particular protein-binding molecules on its surface. These molecules can be anything from antibodies to small-molecule ligands, but regardless, they are selected or designed such that they bind with high specificity to particular proteins that are expressed on the surface of the cancer cells being targeted. Thus, for example, drug-containing microbubbles could be injected into a patient's blood stream, where if they came into contact with cells expressing the

protein being targeted, the microbubbles would stick to those cells. Some external application such as high-frequency ultrasound could then be used to disrupt the microbubbles, releasing the chemotherapy drugs into the immediate vicinity of the cancer cells [2]. As such, many healthy tissues would be spared the destructive effects of the drugs, whose side effects would then be greatly diminished.

In the present work, the first steps were taken towards developing just such a system utilizing peptoids (poly-*N*-substituted glycines) as the targeting compounds, i.e. the protein-binding molecules that would be on the outside of the microbubble. Peptoids are very similar to naturally occurring peptides, except that in peptoids the side chains are attached to the nitrogen instead of the α-carbon (Figure 1). Additionally, the backbone of peptoids is achiral and the molecules do not use hydrogen bonds to form secondary structures. Like peptides and antibodies, peptoids can be designed to act as ligands for particular proteins; however, because of their structural differences from peptides, peptoids are largely protease resistant [3,4]. This makes peptoids promising compounds for use in *in vivo* applications, such as targeting cancer cells.

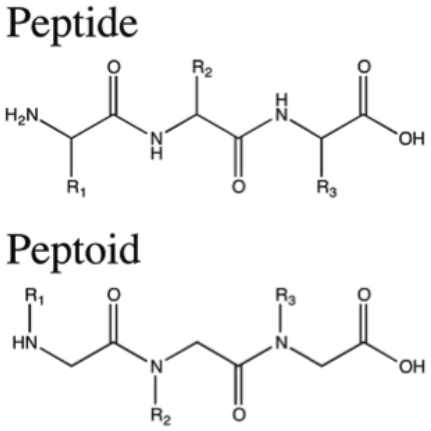

Figure 1. General structures of peptides and peptoids.

A limitation in creating peptoids that specifically target cancer cells is that the proteins on the surface of many cancerous cells are very similar to those of non-cancerous ones. However, recent literature has indicated that lectin-like oxidized low-density lipoprotein receptors (LOX-1) and receptors for advanced glycation end-products (RAGE) are expressed at higher levels during metastasis of certain cancers [5,6]. The increased expression of these membrane proteins can be taken advantage of by creating a "bifunctional" peptoid complex that will attach to the combination of LOX-1 and RAGE with high specificity (Figure 2). The work outlined in this paper details efforts to design and optimize a peptoid that acts as a ligand for LOX-1.

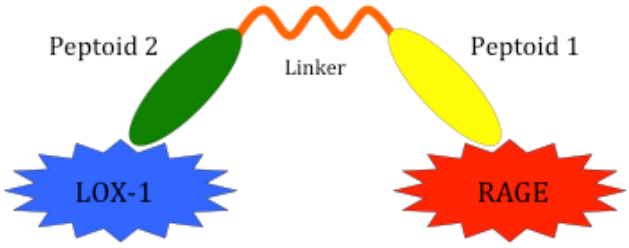

Figure 2. Diagram of the bifunctional peptoid targeting complex.

Methods and Materials

Unfortunately, it is unclear which peptoid chemistries should have the best binding characteristics towards LOX-1. As such, a combinatorial peptoid library was synthesized, which enabled the rapid screening of several thousand different peptoid sequences for LOX-1 binding. Peptoids are synthesized on resin beads by starting with a resin-bound amine group and reacting it with a haloacetic acid (typically bromoacetic acid). The halogen is then replaced with the amine group of a

second “submonomer” (the side chain), thus adding another functional group to the peptoid. The cycle is repeated until the desired peptoid sequence is achieved (Figure 3) [3,4]. All peptoids in the library were synthesized using a modified automated peptide synthesizer.

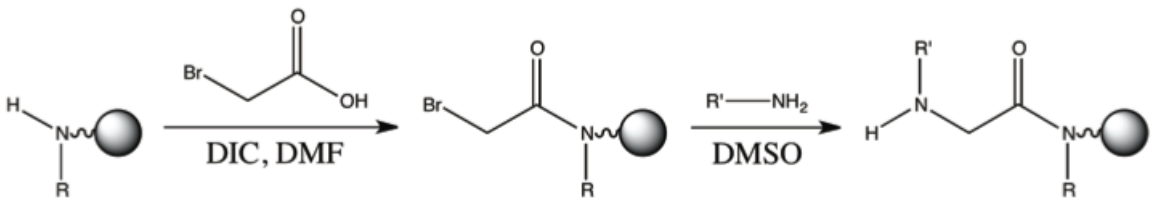

Figure 3. General peptoid synthesis protocol.

The combinatorial peptoid library was created using five different side chains that were chosen based on their performance in a previous study (Figure 4) [4]. The peptoids were synthesized on Tenta-Gel Macrobead® resin using a mix-and-split method such that the library was composed of 6-mer peptoids with every combination of the five side chains (Figure 5). This resulted in a library with a theoretical diversity of 15,625 (or 5^6) compounds.

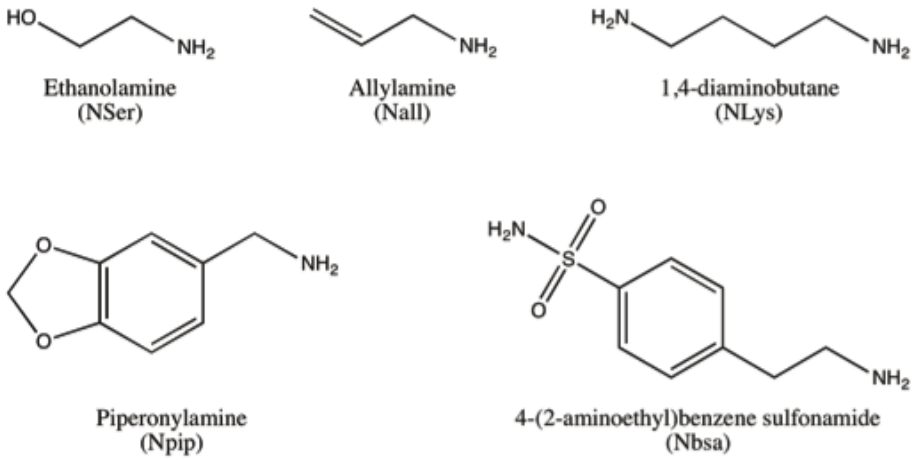

Figure 4. Structures of the five side chains used to build the library.

Figure 5. Diagram of the mix-and-split method used to create the peptoid library.

The peptoids were screened for LOX-1 binding on their respective beads using an adapted enzyme-linked immunosorbent assay (ELISA) protocol (Figure 6). First, nonspecific protein binding was blocked for on the bead using a casein solution. Then the beads were incubated with the extracellular domain of LOX-1. Next, biotinylated anti-LOX-1 antibody was introduced. Finally, the beads were incubated with streptavidin-conjugated AlexaFluor® 647. A fluorescence microscope was used to detect which beads displayed fluorescence, indicating that the biotinylated anti-LOX-1 had bound to them, which implied that LOX-1 binding might have occurred. Beads that displayed high fluorescence could then be isolated to determine the exact peptoid structure on them.

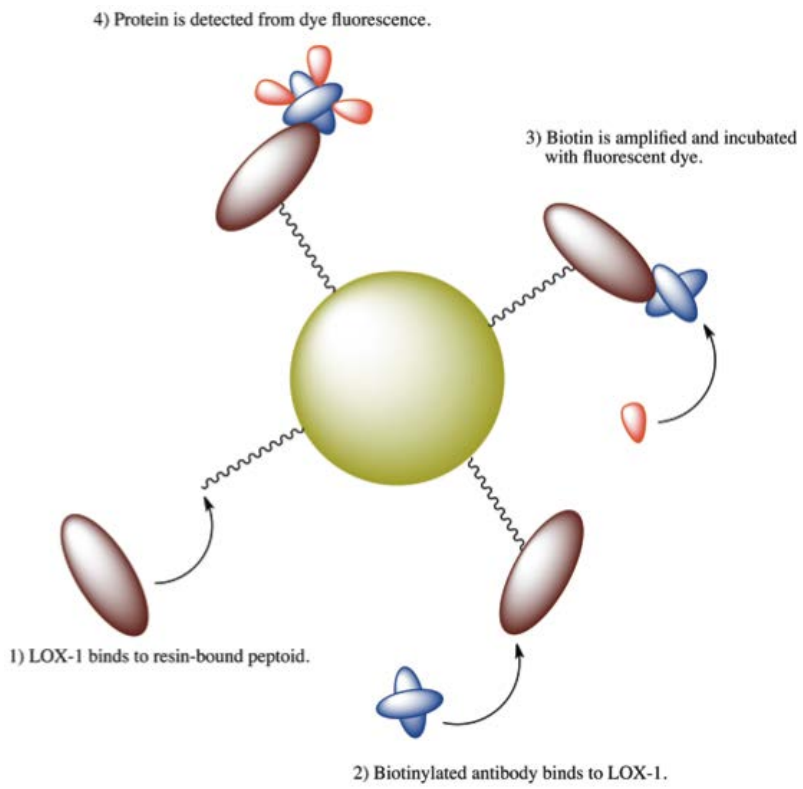

Figure 6. Diagram of the modified ELISA process used to detect LOX-1 binding.

Unfortunately, because of the way the combinatorial library was synthesized it is impossible the know the side-chain sequence, i.e. the chemical structure, of the peptoid on any particular bead simply by looking at the bead—a bead cataloging system was not used because it would have been prohibitively difficult. As such, a sequencing technique must be used to determine the structures of any peptoids that showed potential LOX-1 binding. Specifically, Edman degradation, though usually used for sequencing peptides and proteins, has been shown to work with peptoids, including on-resin peptoids on single beads [4]. Edman degradation procedures were carried out using an automated peptide sequencer. Because the peptoids were not made of amino acids, new standards had to be run before sequencing could

begin. Peptoid trimers were made from each of the five side-chains used in the library in order to run standards.

Results and Discussion

The combinatorial library was successfully synthesized and screened for LOX-1 binding. Of the many beads that displayed fluorescence indicating the potential presence of LOX-1 ligands, ten that displayed relatively large signals were selected and isolated (Figure 7). Work then began on sequencing the selected peptoids. Unfortunately, because of time constraints and technical issues, none of the potential LOX-1 binding peptoids were sequenced as of this writing. However, the side-chain standards were successfully run and their characteristic retention times were determined for the sequencer being utilized (Table 1).

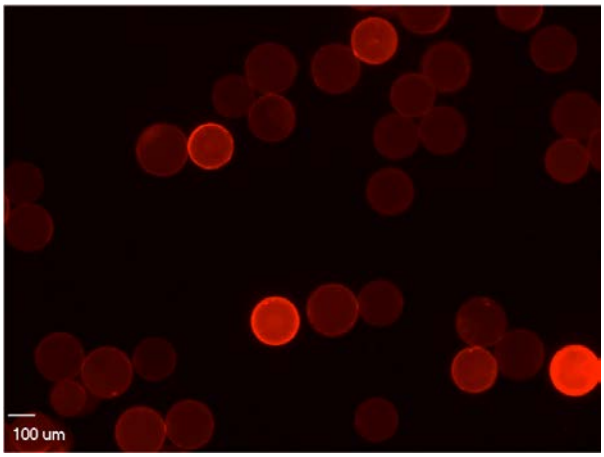

Figure 7. Micrograph of fluorescing resin beads from the LOX-1 affinity screening.

Table 1. Characteristic retention times of the side chains.

Side Chain	NSer	Nbsa	Nall	Nlys	Npip
Retention Time (min)	8.43	15.57	17.09	17.75	20.77

Additionally, an on-resin peptoid of known structure was sequenced to test the automated sequencer. This peptoid was synthesized as part of another project, but it had the sequence Nbsa-NLys-Nbsa-Npip-Nlys, of which all the side chain retention times had been determined as shown above. The results of sequencing this peptoid seemed to agree with the standards; however, there was significant noise due to the extremely low peptoid concentration on only a single resin bead, which illustrates the difficulties of performing this type of sequencing.

Conclusions

A combinatorial peptoid library was synthesized that allowed the rapid screening of thousands peptoid structures for LOX-1 binding. Once the selected peptoids that displayed potential for LOX-1 binding are successfully sequenced, they can be synthesized and cleaved from the resin for use in quantitative binding assays. Those peptoids that display LOX-1 binding can then be further engineered to achieve the desired characteristics.

Eventually this procedure will be repeated for the protein RAGE. Then the peptoid ligands for LOX-1 and RAGE will be combined into a complex to act as a targeting molecule for drug-loaded microbubbles. This technology has great potential for simultaneously increasing the efficacy of cancer chemotherapy treatment while also decreasing said treatment's side effects.

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